1. 35 U.S.C. §112 Rejections

a. 35 U.S.C. §112, first paragraph

Claims 58-172 have been rejected under 35 U.S.C. §112, first paragraph because

> the specification, while being enabling for specific combinations of substances (first substance, second substance and third substance), specific liquid medium, does not reasonably provide enablement for claims as claimed with confusing terminology.

The Office further states that claim 58

> recites three amphipipathic substances, but only two of them form extended surfaces, but the other doesn't, but only gets attached to the extended surfaces. One of ordinary skill in the art would not be able to determine which ampipathic substances form extended surfaces and which do not from instant specification which provides lengthy lists of substances which overlap.

Applicant respectfully submits that claim 58 has been amended and complies with 35 U.S.C. §112, first paragraph. Regarding the determination of amphipathic substances, Applicant respectfully submits that, as set out in the application, amphipathic molecules (the third substance), namely the macromolecules or chain molecules already discussed, associate better with an extended surface which comprises at least one amphipathic substance, which tends to form extended surfaces (the first substance), and at least one more substance, which is more soluble in the suspending liquid medium and also which tends to form less extended surfaces (the second substance) than the former amphipathic substance. In other words, the presence of a substance with surface destabilizing tendency renders surface-solution interface relatively more attractive for the adsorbing macromolecules compared with the corresponding surfaces formed from the less soluble surface-forming substance only, in the absence of the formed more soluble, surface destabilizing second substance (See page 14, lines 5-14).

Further, "extended surfaces" are described in the application as follows:

> A surface, in the context of this document, is deemed to be extended if it allows propagation and/or evolution of cooperative surface excitations in two dimensions. The surface of a vesicle, for example, fulfils this criterion by supporting surface undulations or fluctuations; depending on membrane flexibility, average vesicle

> diameters between 20 nm and several hundred nanometers are needed for this. (Mixed) Lipid micelles, which do not reach this dimension at least in one direction, do not fulfill the requirement; if so, their surface is not considered to be extended in the sense of this invention.

Page 14, lines 14-21.

As further set out in the present invention, amphipaths, especially macromolecules (the third substance) adsorb to soft surfaces comprising a mixture of lipids (the first substance) and surfactants (the second substance) more efficiently than to lipid aggregates containing no surface-active molecules. More generally speaking, a blend of molecules forming a stable membrane – typically but not necessarily in the form of lipid vesicles (liposomes) – and at least one strongly amphipathic, that is, relatively water soluble, bilayer-destabilising component (often a surfactant), exemplified by a mixture of phospholipids and surfactants, is more prone to bind amphipaths , such as proteins than pure phospholipid surfaces, especially vesicles or liposomes which consist of phospholipids only or also comprise at least one bilayer stabilizing lipid class substance (See p. 6, lines 8-17).

Further, Applicants respectfully submit that the selection of the first, second and third substances is clearly set out in the present claims and is supported in the application. For example, the first substance and second substance are selected from amphipaths that form extended surfaces in contact with the liquid medium. The solubility of the second substance in the liquid medium is greater than the solubility of the first amphiphatic substance in the liquid medium. Further, the extended surfaces formed by the first substance are greater than the extended surfaces formed by the second substance. (See claim 58) Further, as set out in the application, the first substance is preferably selected from lipids or lipid-like materials (See page 17 line 28 – page 18, line 11). The second substance is preferable an edge-active substance or surfactant (See page 14, lines 23-24; page 18 line 13 – page 19 line 8) The third substance is preferably a chain molecule (See page 19 line 28 – page 20 line 4) and associates with the extended surfaces formed by the first substance and the second substance.

Applicants respectfully submit that the present claims are enabling and that one of skill in the art could readily determine which ampipathic substances form extended surfaces and which ampipathic substances do not form extended surfaces. Accordingly, reconsideration and withdrawal of the 35 U.S.C. §112 rejection is respectfully requested.

b. 35 U.S.C. §112, second paragraph

Claims 58-172 have also been rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as his invention.

Applicants respectfully submit that definiteness of claim language must be analyzed, not in a vacuum, but in light of: (A) The content of the particular application disclosure; (B) The teachings of the prior art; and (C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. See MPEP §2173.02.

In particular, the Office first asks "if a substance is soluble in a liquid (regardless of the degree of solubility) how can it form extended surfaces? What is meant by an extended surface?"

Applicants respectfully submit that "extended surface" is a term well-known to one skilled in the art and is clearly defined in the application as follows:

> A surface, in the context of this document, is deemed to be extended if it allows propagation and/or evolution of cooperative surface excitations in two dimensions. The surface of a vesicle, for example, fulfils this criterion by supporting surface undulations or fluctuations; depending on membrane flexibility, average vesicle diameters between 20 nm and several hundred nanometers are needed for this. (Mixed) Lipid micelles, which do not reach this dimension at least in one direction, do not fulfill the requirement; if so, their surface is not considered to be extended in the sense of this invention.

Page 14, lines 14-21.

Applicants respectfully submit that soluble components may form aggregates or surfaces upon combination or, for example, due to a certain concentration in the liquid medium. Solubility does not automatically exclude formation of aggregates. This is a phenomenon well-known in biochemistry wherein two liquids are combined, which together form a solid or membrane or surface. Household detergents are examples of this phenomenon.

The Office further asks: "If all three are amphipathic substances, why would only two form extended surfaces and the third one not? What is the solubility of the third substance?"

Applicants respectfully submit that the substances are all amphipathic (having both hydrophobic and hydrophilic regions). The first two substances are selected from amphiphatic substances that form extended surfaces. The third is selected from amphiphatic substances that do not form extended surfaces. The substances are selected from different groups (eg. lipids, surfactants or edge-active substances and chain molecules) and, as such, possess different properties and functions.

Applicants further submit that the solubility of the third substance in the liquid medium is not necessarily an important feature of the present invention and, thus, its solubility is not set out nor is it required to be set out in the claims.

The Office further asserts that "'less extended', 'more extended' are indefinite since they are relative terms."

Applicants respectfully disagree. Applicants respectfully submit that the fact that claim language, including terms of degree, may not be precise, does not automatically render the claim indefinite under 35 U.S.C. 112, second paragraph. *See* MPEP 2173.05(b). In the present case, Applicants merely claim that "the solubility of the second amphiphatic substance in the liquid medium being greater than the solubility of the first amphiphatic substance in the liquid medium" and "the extended surfaces formed by the first substance are greater than the extended surfaces formed by the second substance." Thus, Applicants are comparing the solubilities and

amounts of extended surfaces of one claimed substance in relation to the other claimed substance. Applicants' comparisons provide guidelines for one of skill in the art to select appropriate first and second substances. For example, if one selects a first substance having a particular solubility "X", then the second substance must be selected from those substances which have a solubility greater than "X".

The Office further states that "[a]ccording to claim 58, only the first and second substance form the extended surfaces; according to claim 60, even the third substance forms the extended surface. This is very confusing."

Applicants respectfully submit that claims 60-62 have been amended to clarify that only the first and second substance form extended surfaces.

The Office further asks "if all three substances have the same sign, how can they associate with each other?" As set out in the application:

> We have also found that the relative number of bound amphipathic macromolecules (proteins) is unexpectedly higher for the surfaces which bear net charges with the same sign as the net charge of the adsorbing entity. This is a clear contradiction within the published information, which teaches that electrostatic binding requires opposite charges on the interacting entities in order to be strong.

Page 6, lines 17-22.

Thus, contrary to published information, Applicants have found that the present combinations of substances associate with each other even if the substances have the same sign. Further, Applicants respectfully submit that not all of the first, second and third substance are necessarily charged, but, rather, the extended surfaces of the first and second substances are charged.

Claim 61 has been amended to eliminate the confusion regarding usage of the term "both." Claims 67-70 and 172 have been amended to correct typographical errors regarding dependencies to claims that were previously cancelled, without prejudice.

The Office further asks "[w]hat is meant by 'average curvature' as recited in claims 71-74? Average of what?" As set out in the application:

> [T]he selection to be made can also be defined in terms of resulting surface curvatures. Using the above mentioned example of a phospholipid (as the basic surface-forming substance) mixed with a surfactant (as the surface-destabilising, more soluble second ingredient) in water (used as the liquid medium) the resulting vesicles attain some characteristic surface curvature. The (average) curvature is, generally speaking, defined as the inverse average radius if the areas enclosed by the surfaces under consideration.

Page 15, lines 25-31.

The Office further states "According to claim 86, the second and third amphopaths are surfactants and are identical. If so, it is unclear why only the second surfactant forms the extended surface and the third doesn't."

Applicant has amended claim 86 herein for clarity.

For clarity, claims 93-97, 100-101, 111, 124, 128, 131 and 162 have been amended as suggested by the Office.

The Office further asks "[w]hat is the difference between 'glycolipid' and 'a carbohydrate containing lipid' as recited in claim 94?"

Applicants respectfully submit that a glycolipid is a lipid that falls within the general category of carbohydrate containing lipids. Accordingly, claim 94 has been amended accordingly.

For clarity, as suggested by the Examiner, Applicants have amended claims 97 and 101 to delete the term 'types', as suggested by the Office.

The Office further states that "[I]t is unclear whether the terms in parenthesis are indeed the limitations recited in claim 105." Applicants respectfully submit that Arlacel and Span are trademarks for specific surfactant types. In particular, Arlacel-type surfactants are generally sorbitane based fatty acid esters such as Arlacel 20-85, glyceryl sorbitane fatty acid esters (Arlacel 481, 986), or polyoxyethylene glyceryl sorbitane fatty acid esters (Arlacel 581, 582, 988). Span-type surfactants, as well, are sorbitane based fatty acid esters. Applicants have amended claim 105 accordingly.

The Office further asserts:

> The method of preparing claim 157 is very confusing; what is meant by 'third substance in the form of said agent molecules'? What is a controlled mechanical fragmentation? Isn't the method for associating the third substance with the first substance and the second? How can this be fragmentation?

For clarification, Applicants have amended claim 157 and have added claim 173.

Further, mechanical fragmentation is well-known to those skilled in the art and refers to, for example, mechanical operations such as filtration, pressure change or mechanical homogenization, shaking, stirring, mixing, etc. "Fragmentation" means "comminuting", both of which are terms well-known to those skilled in the art, and refers to the mixture of substances by, for example, the above set out means. As set out by Applicants:

> In operating the method, one may advantageously mix the selected ingredients separately and, if required, co/dissolve the components in solution, then combine the resulting mixture(s) or solution(s) and finally to induce the formation of agent-binding entities or surfaces, preferably by the action of mechanical energy, as already explained.

Page 24, lines 24-27.

"Mechanical energy" is described as:

> Preferred methods for preparing invented extended surfaces include mechanical operations on a corresponding mixture of substances, such as filtration, pressure change or mechanical homogenization, shaking, stirring, mixing, or by means of any other controlled mechanical fragmentation in the presence of the agent molecules which are to associate with the surface formed in the process.

Page 23, lines 27-31.

The Office further asks "[w]hat is an 'edge active substance' as recited in claim 161?"

Applicants respectfully submit that, as set out in the application:

> "Edge active" substance or "surfactant", in this application, refers to any substance which increases the system's propensity to form edges, protrusions or other strongly curved structures and defect-rich regions...

See page 10, line 4 – line 20.

The Office further asks "[w]hat is meant by 'recognition molecules', adrenocorticostatica and 'adrenolitica' as recited in claim 162? (similar terms are also recited in claim 126)" and "[w]hat are those terms recited in claim 126?"

Applicants respectfully submit that the terms originally recited in claims 162 and 126 were incorrect due to translational differences and have been amended in accordance with the correct terminology.

"Recognition molecule" is a term well-known to those skilled in the art and refers to single molecules or small complexes of molecules that are capable of specifically recognizing or binding to some practically relevant targets, which can be a molecule, a molecular complex, a cell organelle (e.g. a membrane) or a whole cell. Examples of recognition molecules are various adhesion proteins, antibodies (members of the immunoglobulin superfamily), biotin or avidin,

chaperones, chaperone related ATP's, ICAM's ICAM related neuronal glycoproteins, (oligo)nucleotides such as RNA and DNA, certain toxins or other specific binding molecules found on pathogens.

It is respectfully submitted that the claims comply with 35 U.S.C. §112. Reconsideration and withdrawal of the rejection is respectfully requested.

2. 35 U.S.C. §102 Rejections

Claims 58-172 have been rejected under 35 U.S.C. §102(e) as being anticipated by WO 92/03122. The Office states:

> WO and CA publications disclose a composition containing two or more amphiphilic substances with different solubilities for the administration of various active substances including insulin (not the abstract and the entire publication, in particular, examples and claims in both).

Applicants respectfully traverse this rejection.

The present invention teaches the formation of extended surfaces by a first substance, preferably a lipid, and a second substance, preferably a surfactant, wherein a third macromolecular substance is associated at the extended surfaces.

WO 92/03122 does not describe or otherwise suggest the association of macromolecules to extended surfaces, as required by the claims of the present invention. Rather, WO 92/03122 exclusively refers to the incorporation of active agents into the described "transfersomes" and their subsequent transport through natural barriers like the skin. WO 92/03122 does not describe or otherwise suggest the association, i.e. adsorption, of macromolecules specifically at extended surfaces as taught by the present invention.

Rather, according to the WO 92/03122 reference, droplets are formed from a solution containing the active agent. This automatically leads to encapsulation of the active agent in the droplet surrounded by the membrane. The agent solution then just forms the "filling" of the

vesicle. Since the agent is dissolved, it will not associate with the membrane in any relevant amount. In the present invention, on the other hand, the agent is bonded or associated to the membrane itself.

CA 2052164, likewise, does not describe or otherwise suggest the association of macromolecules to extended surfaces, as required by the claims of the present invention. Rather, CA 2052164 exclusively refers to the incorporation of molecules into liposomes, emulsions or micelles. This is actually contradictory to the teachings of the present invention. Further, surfactants described by CA 2052164 are specified as those capable of forming micelles, such as polyoxyethylene sorbitan fatty acid esters, sodium salts of fatty acids, or polyoxyethylene hydrogenated castor oil. The use of surfactants for the formation of micelles is exclusive to their use as additives, not as a membrane forming component as taught by the present invention. Thus, the CA 2052164 does not describe or otherwise suggest that surfactants form extended surfaces together with the lipids. Thus, the combination of lipids and surfactants for the formation of extended surfaces is not described or otherwise suggested by CA 2052164. Indeed, on page 6, there is a clear distinction between the preparation of liposomes from membrane-forming components and the preparation of micelles from micelle-forming surfactants. Further, as an active agent, CA 2052164 mentions high molecular drugs like insulin and polysaccharides and explicitly describes these agents as being encapsulated in the liposomes. (see page 6) Still further, in the examples, only 6-carboxyfluorescine (6-CF), which is not a macromolecular substance, is mentioned.

As provided in MPEP-2131, a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegal Bros. v. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Or stated another way, "The identical invention must be shown in as complete detail as is contained in the ... claims. *Richardson v Suziki Motor Co.*, 868 F.2d 1226, 9 USPQ 2d. 1913, 1920 (Fed. Cir. 1989). Although identify of terminology is not required, the elements must be arranged as required by the claim. *In re Bond*, 15 USPQ2d 1566 (Fed. Cir. 1990).

It is clear from the foregoing remarks that the above- identified claims are not anticipated by the WO 92/03122 or the CA 2052164 reference. Namely, the references fail to describe or otherwise suggest the association of macromolecules to extended surfaces, as required by the claims of the present invention.

CONCLUSION

Reconsideration and allowance of claims 58-193 is respectfully requested in view of the foregoing discussion. This case is believed to be in condition for immediate allowance. Applicant respectfully requests early consideration and allowance of the subject application.

If for any reason a fee is required, a fee paid is inadequate or credit is owed for any excess fee paid, you are hereby authorized and requested to charge Deposit Account No. **04-1105**.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned attorney would appreciate the opportunity to do so.

Respectfully submitted,

Date: 2/13/03

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VERSION WITH MARKINGS TO SHOW CHANGES MADE IN CLAIMS

Please note that additions to the claims are shown underlined and deletions are shown in brackets.

58. A combination of substances in a liquid medium comprising:

at least one first amphiphatic substance selected from lipids; [and]

at least one second amphiphatic substance selected from edge-active substances, surfactants and combinations thereof; and

at least one third amphiphatic substance selected from chain molecules;

wherein the first substance and the second substance [of which exhibit amphipathic properties when contacted in a suitable liquid medium, and] form extended surfaces in contact with the medium, [the molecules of at least one third amphipathic substance associating with the extended surfaces,]

wherein the solubility of the second amphiphatic substance in the liquid medium is greater than the solubility of the first amphiphatic substance in the liquid medium,

[the at least one first and at least one second substance have different solubilities in the liquid medium, the at least one first substance is less soluble in said liquid medium acting as the surface building substance,

the at least one second substance is more soluble in said liquid medium,]

wherein the extended surfaces formed by the first substance are greater than the extended surfaces formed by the second substance,

[forming less extended surfaces than the at least one first substance alone,]

wherein the extended surfaces formed by the first substance and the second substance combined are more extended than the extended surfaces formed by the first substance alone,

wherein molecules of the third substance associate with the extended surfaces formed by the first substance and the second substance, [and]

wherein the presence of said at least one second substance in the combination increases the ability of the molecules of the third substance to associate [leads to an increase of the

association of said at least one third substance] with the extended surfaces formed by the at least one first substance and the at least one second substance.

60. The combination of claim 58 wherein the extended surfaces[,] formed by the first[,] and second substance carry a net electric charge and wherein the third substance[s] [carry] carries a net electric charge, the molecules of the third substance associating with the extended surfaces, and the net charge density of the surfaces and the net charge of the molecules associating with the surfaces having the same sign.

61. The combination of claim 58 wherein the extended surfaces formed by the first[,] and second substance are negatively charged and wherein the third substance[s are both] is negatively charged.

62. The combination of claim 58 wherein the extended surfaces formed by the first[,] and second substance are positively charged and wherein the third substance[s are both] is positively charged.

67. The combination of claim 64 wherein the concentration of the second substance is at least 0.1 % of the relative concentration as defined in claim [8] 66.

68. The combination of claim 64 wherein the concentration of the second substance is from 1 to 80 percent of the relative concentration as defined in claim [8] 66.

69. The combination of claim 64 wherein the concentration of the second substance is from 10 to 60 percent [at least 0.1 %] of the relative concentration as defined in claim [8] 66.

70. The combination of claim 64 wherein the concentration of the second substance is from [1 to 80] 20 to 50 percent of the relative concentration as defined in claim [8] 66.

71. The combination of claim [64] 66 wherein the surfaces have an average curvature, yielding an average radius between 15 nm and 5000 nm.

72. The combination of claim [64] 66 wherein the surfaces have an average curvature, yielding an average radius between 30 nm and 1000 nm.

73. The combination of claim [64] 66 wherein the surfaces have an average curvature, yielding an average radius between 40 nm and 300 nm.

74. The combination of claim [64] 66 wherein the surfaces have an average curvature, yielding an average radius between 50 nm and 150 nm.

75. The combination of claim [64] 66 wherein the surface is supported by a solid.

86. The combination according to claim 60 wherein the first substance is less soluble in the liquid medium, and/or being the surface-building and/or charge carrying amphipatic substance in the system, is a lipid, whereas the second substance is more soluble in the liquid medium, and/or causing increased surface curvature, flexibility or adaptability and/or being the charge carrying substance, is a surfactant[, or is identical with the third substance].

93. The combination of claim 58 wherein the first extended surfaces forming substance is selected from the group [comprising] consisting of lipids, lipids from a biological source, corresponding synthetic lipids, and modifications of such lipids.

94. The combination of claim 93 wherein the first extended surfaces forming substance is selected from the group [comprising] consisting of glycerides, [glycolipids,] glycerophospholipids, isoprenoidlipids, sphingolipids, steroids, sterines or sterols, sulphur-containing lipids, a carbohydrate-containing lipids and half-protonated fluid fatty acids.

95. The combination according to claim 93 wherein the first extended surfaces forming substance is selected from the group [comprising] consisting of phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins, sphingophospholipids, glycosphingolipids, cerebrosides, ceramidpolyhexosides, sulphatides,sphingoplasmalogenes, and gangliosides.

96. The combination according to claim 93 wherein the first extended surfaces forming substance is selected from the group [comprising] consisting of diacyl-, dialkenoyl- and dialkyl-lipids.

97. The combination according to claim 93 wherein the first extended surfaces-forming substance is selected from the group [comprising] consisting of [lipids of the] dioleoyl-lipids, dilinoleyl- lipids, dilinolenyl- lipids, dilinolenoyl- lipids, diarachidoyl- lipids, dilauroyl-lipids, dimyristoyl- lipids, dipalmitoyl- lipids, distearoyl- lipids, [or corresponding] and sphingosine- lipids [derivative types].

100. The combination of claim 98 wherein the surfactant is selected from the group [comprising] consisting of nonionic, zwitterionic, anionic and cationic surfactants.

101. The combination of claim 98 wherein the surfactant is selected from the group, [comprising] consisting of long-chain fatty acids or long-chain fatty alcohols, alkyltrimethyl-ammonium salts, [dialkyldimethyl] alkyldimethyl-ammonium salts, [trialkylmethyl] alkylmethyl-ammonium salts, alkylsulphate salts, monovalent salts of cholate, deoxycholates, glycocholates, glycodeoxycholates, taurodeoxycholates, taurocholates, acyl dimethyl-aminoxides, alkanoyl dimethyl-aminoxides, dodecyl dimethyl-aminoxide, alkyl-N-methylglucamides, alkanoyl-N-methylglucamides, N-alkyl-N,N-dimethylglycines, 3-(acyldimethylammonio)-alkanesulphonates, N-acyl-sulphobetaines, polyethylen-glycol-octylphenyl ethers, nonaethylen-glycol-octylphenyl ether, polyethylene-acyl ethers, nonaethylen-dodecyl ether, polyethyleneglycol-isoacyl ethers, octaethyleneglycol-isotridecyl ether, polyethylene-acyl ethers, octaethylenedodecyl ether, polyethyleneglycol-sorbitane-acyl esters, polyethylenglykol-20-

monolaurate (Tween 20), polyethylenglykol-20-sorbitan-monooleate (Tween 80), polyhydroxyethylene-acyl ethers, polyhydroxyethylene-lauryl ethers, polyhydroxyethylene-myristoyl ethers, polyhydroxyethylene-cetylstearyl ethers, polyhydroxyethylene-oleoyl ethers, polyhydroxyethylen-4, or 6, or 8, or 10, or 12-lauryl ethers (Brij series), or in the corresponding esters, polyhydroxyethylen-8-stearate (Myrj 45), polyhydroxyethylen-laurates [types], polyhydroxyethylen-oleates [types], polyethoxylated castor oil 40 (Cremophor EL), sorbitane-monoalkylates [(Arlacel or Span series)], sorbitane-monolaurate [(Arlacel 20, Span 20)], acyl-N-methylglucamides, alkanoyl-N-methylglucamides, decanoyl-N-methylglucamide, dodecanoyl-N-methylglucamide, alkyl-sulphates, alkyl sulphate saltslauryl-sulphate, oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, fatty acid salts, sodium elaidate, sodium linoleate, sodium laurate, lysophospholipids, n-octadecylene-glycerophosphatidic acid, octadecylene-phosphorylglycerol, octadecylene-phosphorylserine, n-acyl-glycero-phosphatidic acids, lauryl glycero-phosphatidic acids, oleoyl-glycero-phosphatidic acid, n-acyl-phosphorylglycerol, lauryl-phosphorylglycerol, oleoyl-phosphorylglycerol, n-acyl-phosphorylserine, lauryl-phosphorylserine, oleolyl-phosphorylserine, n-tetradecyl-glycero-phosphatidic acid, n-tetradecyl-phosphorylglycerol, n-tetradecyl-phosphorylserine, corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipids, corresponding short-chain phospholipids, and surface-active polypeptides.

105. The combination of claim 58 wherein the surface-supporting at least one first substance is a phosphatidylcholine and/or a phosphatidylglycerol and the at least one second substance less capable of forming the extended surface is a lysophospholipid, a lysophosphatidic acid, methylphosphatidic acid, lysophosphatidylglycerol, lysophosphatidylcholine, a partially N-methylated lysophosphatidylethanolamine, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, or a sufficiently polar sterol derivative, a laurate, myristate, palmitate, oleate, palmitoleate, elaidate or other fatty acid salt and/or a Tween-, a Myrj-, or a Brij-surfactant, or a Triton, a fatty acid sulphonate, -sulphobetaine, -N-glucamide or -sorbitane [(Arlacel or Span)] surfactant.

111. The combination of claim 110 wherein the at least one third substance associating with the extended surface is a chain molecules, selected form the group [comprising] consisting of oligomers or polymers.

114. The combination of claim [53] 111 wherein the chain molecules have an average molecular weight above 1500 Daltons.

124. The combination of claim 123 wherein the polynuleotides are selected from the group [comprising] consisting of DNA and RNA, in the natural form or after chemical, biochemical, or genetic modification.

126. The combination of claim 58 wherein the third substance acts as an [adrenocorticostaticum] adrenocorticoid, a [ß-adrenolyticum] ß-adrenolyte, an androgen an antiandrogen, an [antiparasiticum] antiparasite, an [anabolicum] anabolic, an [anaestheticum] anaesthetic, an [analgesicum] analgesic, an [analepticum] analeptic, an [antiallergicum] antiallergenic, an [antiarrhythmicum] antiarrhythmic, an [antiarteroscleroticum] antisclerotylosis, an [antiasthmaticum] antiasthmatic, a [bronchospasmolyticum] bronchospasmolytic, an [antibioticum] antibiotic, an [antidrepressivum] antidrepressant, an [antipsychoticum] antipsychotic, an [antidiabeticum] antidiabetic, an [antidot] antidote, an [antiemeticum] antiemetic, an [antiepilepticum] antiepileptic, an [antifibrinolyticum] antifibrinolytic, an [anticonvulsivum] anticonvulsive, an [anticholinergicum] anticholinergic, an enzyme, a coenzyme or corresponding inhibitor, an [antihistaminicum] antihistamine, an [antihypertonicum] antihypertensive agents, a biological inhibitor of drug activity, an [antihypotonicum] antihypotensive, an anticoagulant, an [antimycoticum] antimycotic, an [antimyasthenicum] antimyasthenic, an agent against [Morbus] Parkinson or [Morbus] Alzheimer, an [antiphlogisticum] antiphlogistic, an [antipyreticum] antipyretic, an [antirheumaticum] antirheumatic, an [antisepticum] antiseptic, a respiratory [analepticum] analeptic or a respiratory stimulant, a [broncholyticum] anti-bronchitis agent, a [cardiotonicum] cardiotonic, a [chemotherapeuticum] chemotherapeutic agent, a coronary [dilatator] dilator, a [cytostaticum] cytostatic, a [diureticum] diuretic, a [ganglium] ganglion-blocker, a

glucocorticoid, an [antiflew] anti-influenza agent , a [haemostaticum] haemostatic, a [hypnoticum] hypnotic, an [immunoglobuline] immunoglobulin or its fragment, an immunologically active substance, a bioactive carbohydrate, a bioactive carbohydrate derivative, a contraceptive, an anti-migraine agent, a [mineralo-corticoid] mineralocorticoid, a morphine-antagonist, a muscle relaxant, a [narcoticum] narcotic, a [neurotherapeuticum] , neurotherapeutic, a [neurolepticum] neuroleptic, a neurotransmitter or its antagonist, a peptide, a peptide derivative,an [opthalmicum] ophthalmic agents, a [sympaticomimeticum] sympathomimetic agent or a [sympathicolyticum] sympatholytic agent, a para-[sympaticomimeticum] sympathomimetic agent or a para-[sympathicolyticum] sympatholytic agent, a protein, a proteine derivative, a psoriasis drug, a [neurodermitis] neurodermatitis drug, a [mydriaticum] mydriatic, a psychostimulant, rhinologicum, a sleep-inducing agent or its antagonist, a sedating agent, a [spasmolyticum] spasmolytic, [tuberculostaticum] tuberculostatic , [urologicum] urologic agent, a vasoconstrictor or [vasodilatator] vasodilator agents, a [virustaticum] antiviral, a wound-healing substance, or a combination thereof.

128. The combination of claim 58 wherein the third substance has immunomodulating properties, and is selected from the group [comprising] consisting of antibodies, cytokines, lymphokines, chemokines and correspondingly active parts of plants, bacteria, viruses, pathogens, immunogens, or parts or modifications thereof.

131. The combination of claim 58 wherein the third substance is a recognition molecule, selected from the group [comprising] consisting of adherins, antibodies, catenins, selectins, chaperones, or parts thereof.

143. The combination of claim [138] 183 wherein the interferon is selected from the group comprising Interferon alpha, beta and gamma.

144. The combination of claim [138] 183 wherein the composition contains up to 20 relative wt-% interferon.

151. The combination of claim [145] 58 wherein the third substance is immunoglobulin (Ig).

157. A method of preparing a combination according to claim 58 in the form of a formulation of a biologically, cosmetically and/or pharmaceutically active agent, comprising:

selecting the at least one first and the at least one second substance,

forming extended surfaces, when the first and second substances are combined in contact with said medium,

selecting the at least one third substance,

allowing the molecules of the third substance to associate with the [such that said] extended surfaces formed by the at least one first and the at least one second substance [attract and associate with the at least one third substance, being said active agent, to a greater extent than the surfaces formed by the at least one first substance alone and are more extended than the surfaces formed by the at least one first substance alone

generating said combination of surface-forming at least one first and at least one second substances by means of , in the presence of the at least one third substance in the form of said agent molecules, such that said agent molecules associate with said extended surface formed by controlled mechanical fragmentation].

158. The method of claim [157] 173 or 174 wherein the means of controlled mechanical fragmentation are selected from the group comprising filtration, pressure change or mechanical homogenisation, shaking, stirring, and mixing.

161. A method for the preparation of a formulation for non-invasive application of active agents, wherein surfaces capable of associating with the active agent are formed from [an] at least one first substance being an amphiphilic substance, [an] at least one hydrophilic fluid, [an] at least one second substance being an edge active substance or surfactant, and at least one third substance being said active agent [agent and, in case, other customary ingredients, which together form said formulation by], wherein the method comprises separately mixing the at least one first substance [being an amphiphilic substance], the at least one second substance [being an

edge-active substance or a surfactant], the at least one hydrophilic fluid and the at least one third substance [being said active agent], followed by combining the resulting mixtures [then being combined] to subsequently induce the formation of the [entities] surfaces which associate with the active agent [molecules].

162. The method of claim 161 wherein the active agent is selected from the group [comprising] consisting of anti-diabetic agents, growth factors, immunomodulators, enzymes, recognition molecules, [adrenocorticostatica] adrenocorticoid, and [adrenolitica] adrenolyte.

169. The method of claim [163] 158 wherein several filters are used sequentially or in parallel.

172. A method for providing a pharmaceutical composition comprising:

in a combination of claim [1] 58, providing the extended surfaces in the form of membranes formed by the at least one first substance and the at least one second substance surrounding miniature droplets, wherein the at least one third substance being a drug associates with said droplet surface to be carried by said droplets to the place where the drug is intended to act.

Kindly add the following new claims:

173. The method of claim 157 further comprising: utilizing controlled mechanical fragmentation to form extended surfaces.

174. The method of claim 157 further comprising the step of generating the extended surfaces by means of controlled mechanical fragmentation in the presence of the third substance or before the addition of the third substance, such that the third substance associates with the extended surfaces formed by controlled mechanical fragmentation.

175. The combination of claim 65 wherein the concentration of the second substance is at least 0.1 % of the relative concentration as defined in claim 66.

176. The combination of claim 65 wherein the concentration of the second substance is from 1 to 80 percent of the relative concentration as defined in claim 66.

177. The combination of claim 65 wherein the concentration of the second substance is from 10 to 60 percent of the relative concentration as defined in claim 66.

178. The combination of claim 65 wherein the concentration of the second substance is from 20 to 50 percent of the relative concentration as defined in claim 66.

179. The combination of claim 66 wherein the concentration of the second substance is at least 0.1 % of the relative concentration as defined in claim 66.

180. The combination of claim 66 wherein the concentration of the second substance is from 1 to 80 percent of the relative concentration as defined in claim 66.

181. The combination of claim 66 wherein the concentration of the second substance is from 10 to 60 percent of the relative concentration as defined in claim 66.

182. The combination of claim 66 wherein the concentration of the second substance is from 20 to 50 percent of the relative concentration as defined in claim 66.

183. The combination of claim 58, wherein said at least one third substance is interferon being suitable for use in humans or animals.

184. The combination of claim 183, wherein interferon is included in an amount ranging from about 0.1 to about 15 mg interferon/mL.

185. The combination of claim 183, wherein interferon is included in an amount ranging from about 1 to about 10 mg interferon/mL.

186. The use of the combination of claim 58 for medicinal or biological applications.

187. The use of the combination of claim 58 for the preparation of drug carriers or drug depots.

188. The use of the combination of claim 58 for bioengineering or for genetic manipulations.

189. The use of the combination of claim 58 for separation technology, (bio)processing or diagnostic purposes.

190. The use of the combination of claim 58 to stabilize surface-associating molecules and/or in catalysing processes which involve molecules in the surface-associated state.

191. The use of the combination of claim 190 for chain molecules that are at least partially amphipathic.

192. The use of the combination of claim 191, wherein the chain molecules are proteins, polypeptides, polynucleotides or polysaccharides.

193. The use of the combination of claim 58 to affect the kinetics and/or the reversibility of association or dissociation between surface-associating molecules and a complex, adaptable surface.

VERSION WITH MARKINGS TO SHOW CHANGES MADE IN SPECIFICATION

Please note that additions to the specification are shown underlined and deletions are shown in brackets.

Page 1, lines 1-4:

Method for developing, testing and using associates of macromolecules and complex aggregates for improved payload and controllable de/association rates

[The present application claims the benefit of International Application No. PCT/JP99/00191, filed on January 20, 1999.] The present application claims the benefit of International Application No. PCT/EP98/06750, filed on October 23, 1998.

Page 9, line 1, before "DEFINITIONS", please add the following:

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates insulin adsorption on different ultra-deformable vesicles. Transfersomes, as a function of protein/lipid concentration ratio in the bulk. In the lower panel, absolute bound protein amount is shown. In the upper panel, relative amount of vesicle associated insulin is given, no short-term time-effect being observed in either case. (Examples 1-27 A)

Fig. 2 presents the results of insulin binding experiments with ultra-deformable Transfersomes containing cholate as a function of total lipid concentration in the bulk. (Examples 1-27 B)

Fig. 3 gives data on insulin binding to Transfersomes, with cholate as a membrane softener, as a function of relative protein/lipid concentration in the bulk and of binding (incubation) time. (Examples 1-27 C)

Fig. 4 exemplifies insulin association with (binding to) surfactant (cholate or Tween 80) containing Transfersomes, as a function of protein/lipid concentration ratio in the bulk. Absolute and relative amounts of bound protein is shown in the lower and upper panel, respectively, highlighting the effect of changing vesicle composition in case of dilution with a buffer without added cholate. Such a dilution is not influential when Transfersomes contain the less soluble Tween 80. (Examples 46-59)

Fig. 5 provides the data that supports the view that insulin from a solution or protein powder (lyophilisate) binds with comparable efficiency to different Transfersome quantities. (Examples 72-76)

Fig. 6 compares the relative efficiency of insulin binding to conventional liposomes (SPC), to charged liposomes (SPC/SPG) and to charged Transfersomes (SPC/SPG/Tween 80). (Examples 77-92)

Fig. 7 illustrates the effect of increasing surface charge density, created by incorporating increasing relative amounts of charged phospholipid SPG into originally uncharged SPC/Tween (SPC/Tw) Transfersomes, on insulin association with extended surfaces of resulting vesicles. (Examples 96-100)

Fig. 8 offers information on insensitivity of insulin binding to the method used to manufacture ultradeformable vesicles. Transfersomes, as evidenced by the relatively constant relative amount of surface (vesicle) associated protein. (Examples 101-104)

Fig. 9 explores the effect of ultra-deformable vesicle composition (SPC + cholate; SPC + Tween 80), of insulin kind/source (human recombinant insulin in Actrapid solution; lyophilized human insulin; porcine insulin in solution) of association (incubation) time (2 hours to 5 weeks) using plain SPC liposomes as negative control.

Fig. 10 illustrates binding of a larger protein, interferon alpha, on non-ionic (SPC/Tw80) and anionic (SPC/NaChol) ultradeformable vesicles as a function of protein/lipid concentration ratio in the bulk. (Examples 111-134)

Fig. 11 provides evidence for biological activity of insulin delivered transdermally with the aid of charged, highly adjustable lipid vesicles comprising a mixture of a phospholipid (SPC) and of an anionic biosurfactant (cholate), such that ensures original insulin binding to the extended vesicle surface. Change in the blood glucose level after insulin application on the skin at relative time zero directly reflects the effect of insulin in vivo.

Fig. 12 points to the effect of batch-to-batch variability for insulin from the same manufacturer in case of transdermal delivery of the drug in Transfersomes (Transfersulin) in vivo. Open symbols give the result of negative control experiment.

Transfersomes comprising
SPC+SPG/Tween = L/D = 2/1

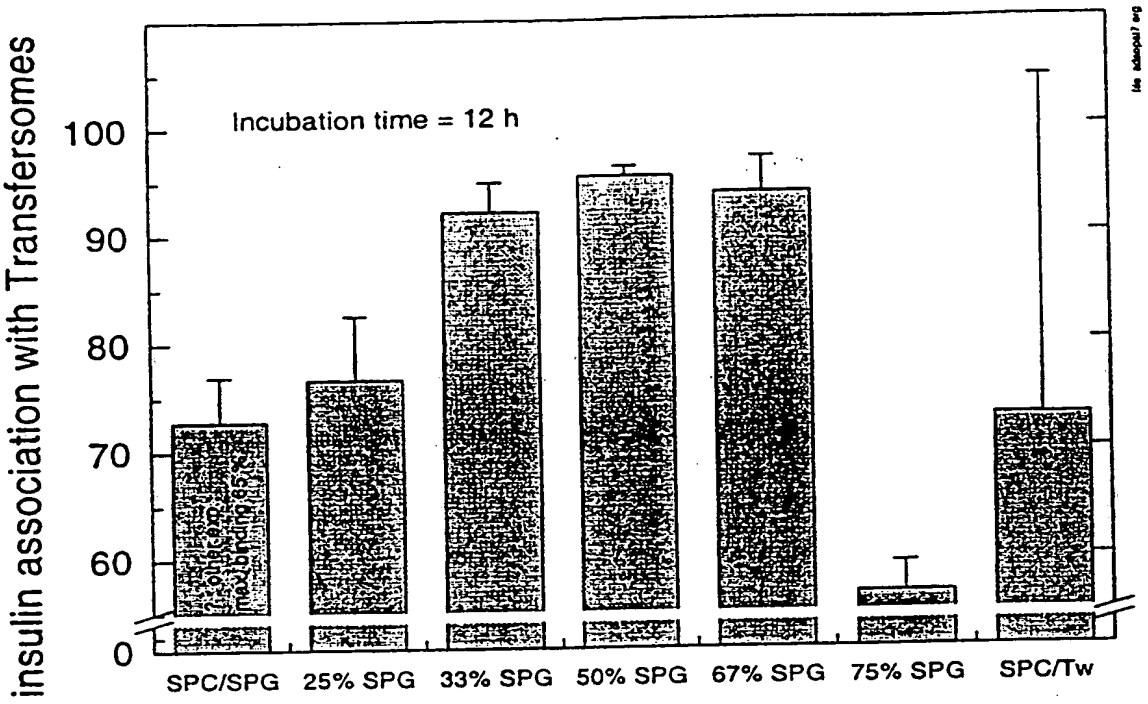

Fig. 7

examples ~~96-98~~ 96-100

Insulin association with Transfersomes C

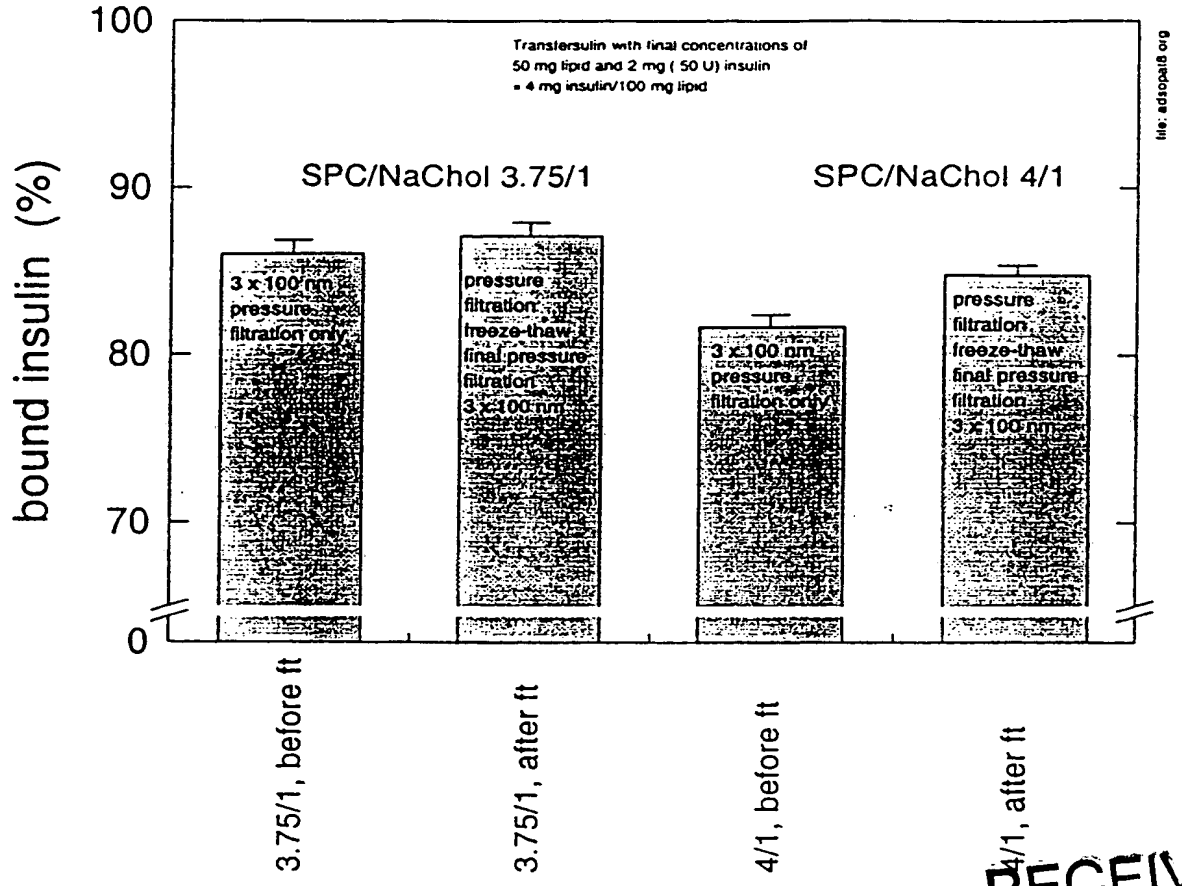

Fig. 8

examples 99-~~100~~ 101-14